Oct 25, 2022 Version 2

Immunostaining on Paraffin Sections of Fly Heads V.2

In 1 collection

DOI
dx.doi.org/10.17504/protocols.io.81wgby61nvpk/v2

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Protocol Citation: Mel Feany 2022. Immunostaining on Paraffin Sections of Fly Heads. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.81wgby61nvpk/v2**Version created by **Daniel El Kodsi**

Protocol status: Working
We use this protocol and it's working

Created: October 25, 2022

Last Modified: May 31, 2024

Protocol Integer ID: 71787

Keywords: ASAPCRN, paraffin sections of fly head, fly head, paraffin section, head, protocol

Abstract

This protocol describes how to perform immunostaining on paraffin sections of fly heads.

Troubleshooting

1 Fix fly heads overnight in 4% formalin.

2 Embed in paraffin.

3 Cut 4 micron sections. Dry at 42 °C Overnight .

4 Deparaffinize and bring through ethanols to water (xylene x 2, 100% ethanol x 4, tap H_2O x 2).

5 Microwave slides in [M] 10 millimolar (mM) sodium citrate for 00:15:00 . 35m
Cool 00:20:00 .

Stock: [M] 100 millimolar (mM) sodium citrate, pH 6.0
Use at least 1 L of citrate solution in large glass box to avoid drying.

6 Block in PBST (PBS with 0.3% Triton) with 2% dry milk for 00:30:00 to 1h 30m
Stock: 10X PBS

7 Incubate with primary antibody Overnight at Room temperature .

8 Wash 3 x in PBST.

9 Incubate with appropriate biotinylated secondary (for immunohistochemistry) or fluorescent secondary (for immunofluorescence) antibody at 1:200 in PBST + milk for 2h
01:00:00 at Room temperature .
For immunohistochemistry incubate in ABC reagent (Vector) for 01:00:00 at Room temperature .

10 Rinse 3 x PBST.

11 Mount slides with antifading medium for immunofluorescence or dehydrate through ethanol series and xylenes and mount in Permount for immunohistochemistry.